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Minireview

MINIREVIEW

The sweet side of the pathogenic *Neisseria*: the role of glycan interactions in colonisation and disease

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One sentence summary: Glycosylation of the surface of meningococci and gonococci, and interactions with glycan structures of the host, are essential for pathogenic *Neisseria* colonisation and disease.

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ABSTRACT

Glycomics is a rapidly growing field that focuses on the structure and function of carbohydrates (glycans) in biological systems. Glycan interactions play a major role in infectious disease, at all stages of colonisation and disease progression. *Neisseria meningitidis*, the cause of meningococcal sepsis and meningitis, and *Neisseria gonorrhoeae*, which causes the sexually transmitted infection gonorrhoea, are responsible for significant morbidity and mortality worldwide. *Neisseria meningitidis* displays a range of surface glycosylations including capsule polysaccharide, lipooligosaccharide and O-linked glycoproteins. While *N. gonorrhoeae* does not have a capsule, it does express both lipooligosaccharide and O-linked glycoproteins. *Neisseria gonorrhoeae* also has the ability to scavenge host sialic acids, while several *N. meningitidis* serogroups can synthesise sialic acid. Surface expressed sialic acid is key in serum resistance and survival in the host. On the host side, the pathogenic *Neisseria* protein adhesins such as Opc and NHBA bind to host glycans for adherence and colonisation of host cells. Essentially, from both the bacterial and host perspective, glycan interactions are fundamental in colonisation and disease of pathogenic *Neisseria*. The key aspects of glycobiology of the pathogenic *Neisseria* are reviewed herein.

Keywords: *Neisseria gonorrhoeae*; *Neisseria meningitidis*; glycomics; carbohydrate; lectin; host–pathogen interactions

INTRODUCTION

Carbohydrates (glycans) and carbohydrate-recognising proteins (lectins) play significant roles in a diverse range of interactions within organisms, as well as between different organisms (Varki and Sharon 2009). Glycans are extremely diverse; there are 10 basic monosaccharide building blocks, and chains of monosaccharides can vary in length, have a range of different linkages and can be linear or branched. These macromolecules are present in all forms of life, and can be found as free oligo- or polysaccharide chains, or as glycoconjugates when attached to proteins or lipids. Protein glycosylation is one of the most common post-translational modifications and enhances the functional diversity of proteins produced by an organism. For example, most mammalian cell surface receptors and almost all serum proteins are glycosylated (Apweiler, Hermjakob and Sharon 1999). Consequently, human cells are covered by a glycocalyx, a rich surface coat of glycans (Varki and Sharon 2009), that is important for mediating interactions with other cells and molecules. Furthermore, glycan interactions between microbes and host cells play a major role in bacterial colonisation and disease (Esko and Sharon 2009).

The pathogenic *Neisseria*, *Neisseria meningitidis* and *N. gonorrhoeae,* display extensive glycosylation of bacterial surfaces

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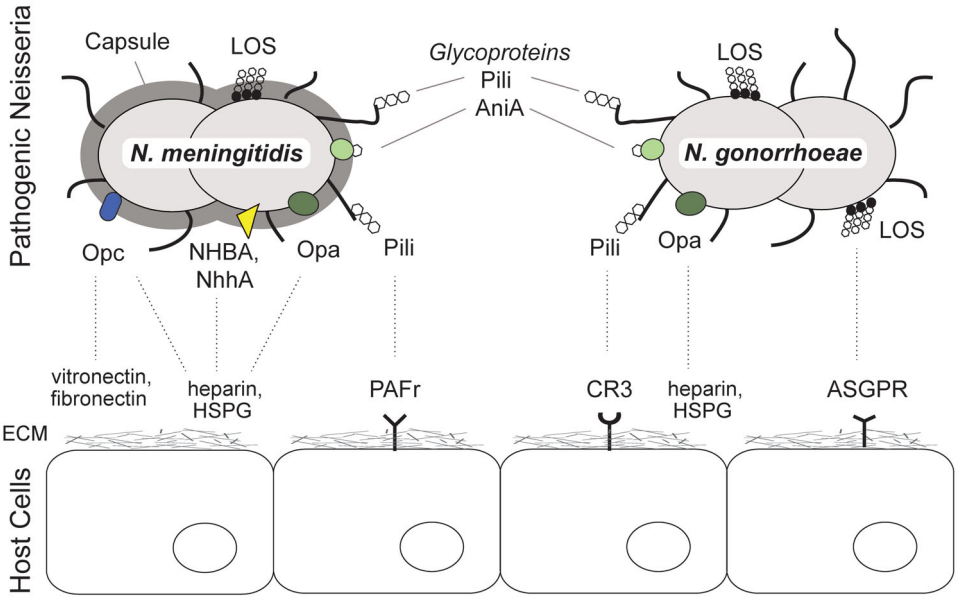

Figure 1. Schematic of surface glycans of the pathogenic *Neisseria*, and glycan-based interactions with host cells. Glycan structures on the surface of the pathogenic *Neisseria* are shown, and include capsule, lipooligosaccharide (LOS), and the glycosylated Pili and AniA proteins. Glycan-dependent interactions between the pathogenic *Neisseria* and host cells are indicated by a dotted line. ECM, extracellular matrix; HSPG, heparan sulfate proteoglycagans; PAFr, platelet activating factor receptor; CR3, complement receptor 3, ASGPR, asialoglycoprotein receptor; NHBA, Neisserial heparin binding antigen.

and structures, and interaction with host glycans is crucial for colonisation and disease. The pathogenic *Neisseria* are closely related obligate human pathogens that share 80%–100% DNA homology, with conservation of the majority of genes (Klee *et al.* 2000). Several of the same bacterial surface structures are involved in initial colonisation of mucosal surfaces by these bacteria (Merz and So 2000); however, they cause distinct diseases. *Neisseria meningitidis* asymptomatically colonises the upper respiratory tract of ∼10%–20% of the population, but also poses a significant health problem due to its ability to cause life-threatening meningitis and septicaemia (Rouphael and Stephens 2012). Despite the availability of effective antibiotics, and vaccines for five serogroups, there are an estimated 1.2 million cases and ∼135 000 deaths attributed to meningococcal disease, each year, worldwide (Rouphael and Stephens 2012). *Neisseria gonorrhoeae*, the causative agent of the sexually transmitted infection gonorrhoea, is a growing global public health threat. Infection typically results in urethritis in males and cervicitis in females; however, asymptomatic cases are common, and untreated gonorrhoea can lead to serious sequelae, including infertility and increased HIV transmission (reviewed in Edwards *et al.* 2016). There are estimated to be more than 106 million cases of gonorrhoea worldwide each year (World Health Organization and Research 2012). Furthermore, there is no available vaccine and the continuing emergence of antibiotic-resistant strains raises concerns that gonorrhoea may become untreatable in the near future (Unemo and Shafer 2014).

In terms of glycosylated surface structures, *N. meningitidis* is a capsulate organism, and both *N. meningitidis* and *N. gonorrhoeae* express lipooligosaccharide (LOS) and glycosylate some of their proteins (Fig. 1). Several of these structures mediate host–pathogen interactions and are involved in virulence. In addition, human host microenvironments are highly glycosylated (Varki, Esko and Colley 2009) and the pathogenic *Neisseria* targets specific host glycan structures for adherence and colonisation of host cells. In this review, the current knowledge regarding the glycans present on the Neisserial surface and the host glycans known to interact with *Neisseria* is discussed. There is a focus on *N. meningitidis*, with key points of difference or interest highlighted for *N. gonorrhoeae*. An overview of the glycan-rich host environments inhabited by the pathogenic *Neisseria* highlights the potential for additional glycan-based interactions during various stages of colonisation and disease.

GLYCAN STRUCTURES OF THE PATHOGENIC NEISSERIA

Meningococcal polysaccharide capsule

The meningococcal capsule is a high molecular weight polysaccharide, which is linked to the bacterial surface by a glycolipid moiety (Tzeng *et al.* 2005). *Neisseria meningitidis* strains can be classified into 13 serogroups, based on structural differences in their capsular polysaccharides (see Table 1). Six serogroups cause the majority of invasive meningococcal disease worldwide (serogroups A, B, C, W135, X and Y) (Rouphael and Stephens 2012). The polysaccharides differ in their individual monomers and/or linkages between these monomers (Harrison *et al.* 2013) (Table 1). Capsule polysaccharide-based vaccines are available for serogroups A, C, W and Y. However, the α(2–8)-linked sialic acid homopolymer of the *N. meningitidis* serogroup B capsule is identical to that produced in mammalian cells (Finne, Leinonen and Mäkelä 1983), and due to this molecular mimicry of host glycans, the serogroup B capsule is poorly immunogenic and has not been used in vaccine development.

The polysaccharide capsule is crucial for invasive meningococcal disease (Spinosa *et al.* 2007), and it is universally expressed by strains isolated from the blood and cerebrospinal fluid (Stephens, Greenwood and Brandtzaeg 2007) The meningococcal capsule increases resistance to the complement-mediated bactericidal activity of human serum, and resistance to opsonophagocytosis (reviewed in Lewis and Ram 2014). Carriage strains isolated from the nasopharynx of healthy individuals may or may not be capsulated (Corbett *et al.* 2004). The

Table 1. Structures of meningococcal polysaccharide capsules.

Serogroup	Glycan repeat unit	Linkage	Description	Ref
A	ManNAc-1-phosphate	α1→6 (phosphodiester)	Homopolymer of α1→6 linked N-acetyl-D-mannosamine-1-phosphate	Liu *et al.* (1971)
B	Neu5Ac	α2→8 (glycosidic)	Homopolymer of α2→8-linked sialic acid	Bhattacharjee *et al.* (1975)
C	Neu5Ac	α2→9 (glycosidic)	Homopolymer of O-acetylated or non-acetylated α2→9-linked sialic acid	Bhattacharjee *et al.* (1975)
D	Not elucidated	–	–	–
E	GalNα3 -7KDO	β2→3 (glycosidic)	Alternating D-galactosamine (GalN) and 2-keto-3-deoxyoctulosonate (KDO) residues	Bhattacharjee *et al.* (1978)
H	Galα1-2 Gro-3-phosphate	Phosphodiester	Partially O-acetylated Galactosyl glycerol-3-phosphate units	Van Der Kaaden *et al.* (1984)
I	LGulNAcα1-3 ManNAcA	β1→4 (glycosidic)	O-Acetylated alternating N-acetyl-guluronic acid and N-acetyl-mannosaminuronic acid residues	Michon *et al.* (1985)
K	ManNAcβ1-4 ManNAcA	β1→3 (glycosidic)	O-Acetylated N-acetylmannosaminuronic acid disaccharide repeat units	Van Der Kaaden *et al.* (1985)
L	GlcNAcβ1-3GlcNAcβ1-3GlcNAc-1-phosphate	Glycosidic and Phosphodiester	Heteropolymer of a trimeric repeating unit	Jennings *et al.* (1983); Litschko *et al.* (2015)
W-135	Galα1-4Neu5Ac	α2→6 (glycosidic)	Heteropolymer of alternating sequences of D-galactose and sialic acid	Bhattacharjee *et al.* (1976)
X	GlcNAc-1-phosphate	α1→4 Phosphodiester	Homopolymer of (α1→4)-linked N-acetylglucosamine-1-phosphate	Bundle *et al.* (1974)
Y	Glcα1-4Neu5Ac	α2→6 (glycosidic)	Heteropolymer of alternating sequences of D-glucose and sialic acid	Bhattacharjee *et al.* (1976)
Z	GalNAcα1 -1Gro-3-phosphate	Phosphodiester	Monosaccharide glycerol-3-phosphate repeat units	Harrison *et al.* (2013)

absence of capsule can be either due to the complete absence of the capsule production genes, known as the capsule-null (*cnl*) phenotype (Claus *et al.* 2002), or due to phase variation (i.e. ON/OFF or graded switching of expression). Two mechanisms of phase variation have been described, slipped strand mispairing of the poly-cytosine tract present within *siaD* and the insertion/excision of the insertion sequence IS1301 in *siaA* (Hammerschmidt *et al.* 1996a,b). In addition, capsule expression is regulated by RNA thermoregulation, with increased temperature upregulating *cssA* translation and consequently, capsule expression (Loh *et al.* 2013). The two-component MisR/MisS system negatively regulates capsule expression, with inactivation of *misR* and *misS* resulting in increased capsule expression (Tzeng *et al.* 2008). The regulation of capsule is reviewed in detail elsewhere (Bartley and Kahler 2014; Tzeng, Thomas and Stephens 2016).

It has been suggested that capsule may block interactions with host cells due to steric hindrance and masking of surface adhesins involved in intimate cellular interactions, as well due to the negative charge of its sialic acid residues (Corbett *et al.* 2004; Virji 2009). Therefore, phase variation and regulation of capsule expression allows the bacteria to adapt to the different stages of infection, i.e. the expression of the capsule is decreased during colonisation but present during invasive disease (Hammerschmidt *et al.* 1996b; Stephens, Greenwood and Brandtzaeg 2007).

Meningococcal and gonococcal LOS

LOS makes up ~75% of the outer membrane of Gram-negative bacteria such as the pathogenic *Neisseria* (Gronow and Brade 2001). Neisserial LOS consists of lipid A that anchors the LOS into the outer membrane, an inner core composed of two 3-deoxy-D-manno-2-octulosonic acid (KDO) and two heptose residues (Hep1 and Hep2 residues), and an outer core of oligosaccharide chains (α, β, γ) attached to the heptose residues (Jennings *et al.* 1999). The individual sugars in each of the oligosaccharide chains are sequentially added onto the elongating chain by

Table 2. Oligosaccharide structures of meningococcal LOS immunotypes.

	α-chain	β-chain	γ-chain	II PEA	Ref
L1	[a]Galα1–4Galβ1–4Glc	–	GlcpNAcα1–2	3P	Di Fabio *et al.* (1990), Griffiss *et al.* (2000)
L2	[a]Galβ1–4GlcNAcβ1–3Galβ1–4Glc	Glcp α1–3	[b]GlcpNAc α1–2	6P	Gamian *et al.* (1992)
L3	[a]Galβ1–4GlcNAcβ1–3Galβ1–4Glc	–	GlcpNAc α1–2	3P	Pavliak *et al.* (1993)
L4	[a]Galβ1–4GlcNAcβ1–3Galβ1–4Glc	–	[b]GlcpNAc α1–2	6P	Kogan *et al.* (1997)
L5	[a]Galβ1–4GlcNAcβ1–3Galβ1–4Glc	Glcp α1–3	[b]GlcpNAc α1–2	–	Michon *et al.* (1990)
L6	[a]4GlcNAcβ1–3Galβ1–4Glc	–	GlcpNAc α1–2	6P	Di Fabio *et al.* (1990)
L7	Galβ1–4GlcNAcβ1–3Galβ1–4Glc	–	GlcpNAc α1–2	6P	Kogan *et al.* (1997)
L8	Galβ1-Glc	–	GlcpNAc α1–2	3P	Griffiss *et al.* (2000)
L9	Galβ1–4GlcNAcβ1–3Galβ1–4Glc	–	GlcpNAc α1–2	6P	Choudhury *et al.* (2008)
L10	Galβ1–4GlcNAcβ1–3Galβ1–4Glc	–	GlcpNAc α1–2	3P/6P	Kim *et al.* (1994)
L11	Glcβ1–4Glc	–	GlcpNAc α1–2	3P&6P	Kim *et al.* (1994), Mistretta *et al.* (2010)

[a]Terminal Gal of α-chain is often partially sialylated.
[b]Partial O-acetylation of the α-D-GlcNAc (of the γ-chain) observed. Phosphoethanolamine (PEA) substituents at O-3 or O-6 of Hep II = 3P or 6P, respectively.

a series of phase-variable glycosyltransferases, which results in variable lengths of the sugar chain within and between strains (Jennings *et al.* 1999). LOS variation between Neisserial species or strains also results from the allelic diversity or presence/absence of glycosyltransferases involved in LOS biosynthesis (reviewed in Bartley and Kahler 2014; Bartley *et al.* 2017). In addition, LOS expression is transcriptionally regulated, with *lgtG* under the control of the MisR/MisS system (Tzeng *et al.* 2008), while *lst* is expression is repressed at low temperatures via an RNA thermosensor (Loh *et al.* 2013). Some of the resulting LOS structures contain a terminal lacto-N-neotetraose (LNnT) (Tsai and Civin 1991), which is a human glycan mimic (Moran, Prendergast and Appelmelk 1996). LNnT may be further modified via the addition of a single sialic acid residue (sialylation). Furthermore, Neisserial LOS can vary during infection. For example, male volunteers challenged with a specific gonococcal isolate which expressed non-sialylated LOS were shown to shed a strain expressing a larger, sialylated LOS type (Schneider *et al.* 1991). LOS variation forms the basis of 12 meningococcal immunotypes (L1–L12, see Table 2); however, there is no LOS typing scheme for *N. gonorrhoeae*.

LOS immunotypes have been linked to meningococcal virulence, and gonococcal LOS plays an important role in pathogenesis and survival. There is a clear role of the sialic acid presence on the LOS in relation to bacterial survival. For example, in *N. meningitidis*, the unsialylated LOS types (L1,8,10) are important for invasion, while the sialylated immunotypes (L3,7,9) are important for survival and dissemination, being less sensitive to complement-mediated killing (Estabrook, Griffiss and Jarvis 1997) and phagocytosis (Unkmeir et al. 2002a) (reviewed in van Putten and Robertson 1995). The presence of a terminal LNnT, resulting from a truncation or deletion of the sialic acid, has been shown to be associated with increased sensitivity to complement (Estabrook, Griffiss and Jarvis 1997).

Similar to *N. meningitidis*, *N. gonorrhoeae's* LNnT structure is essential for LOS-mediated adherence and invasion. Adherence and invasion into male urethral epithelium cells is mediated by the interaction between the gonococcal LOS with the asialoglycoprotein receptor (C-type lectin) (Harvey *et al.* 2001). This interaction is abated in mutants expressing truncated LOS that lack the LNnT structure, and in strains expressing a terminal sialic acid on their LOS (Harvey *et al.* 2001). Gonococcal LOS sialylation decreases non-opsonic, opacity-associated uptake by neutrophils (Rest and Frangipane 1992) and allows direct interaction of gonococcal LOS with factor H (Ram *et al.* 1998), thus promoting serum resistance. Interestingly, LOS sialylation also allows interaction of the pathogenic *Neisseria* with human myeloid cells, via the sialic acid-binding immunoglobulin-like lectins expressed on some phagocytic cells, thereby making the bacteria more susceptible to phagocytosis (Jones, Virji and Crocker 2003).

Glycosylated proteins

Post-translational modification of proteins with glycan moieties (glycosylation) is ubiquitous in nature and is important for protein function. The PilE subunit of pilin and the nitrite reductase AniA are the best characterised Neisserial glycoproteins. The pathogenic *Neisseria* express type IV pili, which are homopolymeric multimers of a 17–21 kDa polypeptide units (pilin/PilE) that can extend several micrometers from the bacterial cell surface. In *Neisseria*, type IV pilins have been grouped in class I or class II pilin. The class I and II pilin genes are present in different genomic locations (Wormann *et al.* 2014): class I pilin (but not class II) is recognised by the SM1 antibody (Virji *et al.* 1989) and class II pilin are restricted to strains from certain clonal complexes (cc1, cc5, cc8, cc11 and cc174) (Wormann *et al.* 2014). These pilin undergo many post-translational modifications, including glycosylation at serine 63 (Stimson *et al.* 1995) and glycosylation of up to five sites on class II pilins (Kahler *et al.* 2001; Gault *et al.* 2015). Pilin glycosylation involves multiple glycosylation (i.e. *pgl*) genes. Of these, the glycosyltransferases *pglA* and *pglE* are subject to phase variation (Jennings *et al.* 1998; Power *et al.* 2003), and consequently the pilin glycan expressed differs across Neisserial strains. For example, pilin can be covalently modified with an O-linked Galβ(1–4)Galα(1–3)-N, N'-diacetylbacillosamine (Gal-Gal-diNAcBac) or Galβ(1–4)Galα(1–3)-glyceramido acetamido trideoxyhexose (Gal-Gal-GATDH) trisaccharide (Chamot-Rooke *et al.* 2007). While a

Gal(α1,3)GlcNAc disaccharide is present in this same position on pili of gonococcal and some meningococcal strains (reviewed in Banerjee and Ghosh 2003; Bartley and Kahler 2014). It has also recently been shown that Gal and GlcNAc residues can be O-acetylated, and O-acetylation impacts oligosaccharide chain length (Anonsen *et al.* 2017).

Neisserial pili are considered to be the primary adhesins involved in early adherence events in colonisation of epithelial cells (Virji 2009). In *N. meningitidis*, pilin glycosylation enhances adherence to epithelial cells (Virji *et al.* 1993), and is required for platelet activating factor receptor-mediated adherence to human airway epithelial cells (Jen *et al.* 2013). In *N. gonorrhoeae,* the glycan on the pili subunit interacts directly with the host complement receptor 3 I-domain that is expressed on the surface of epithelial cells. This interaction facilitates bacterial adherence and promotes intracellular survival (Jennings *et al.* 2011).

The nitrite reductase, AniA, is a glycoprotein that is expressed by all *N. gonorrhoeae* and most *N. meningitidis* strains (Ku *et al.* 2009; Shewell *et al.* 2013). AniA is glycosylated at the C-terminus with the trisaccharide digalactosyl 2,4-diacetamido-2,4,6-trideoxyhexose (Ku *et al.* 2009), which is not required for its nitrite reductase function (Ku *et al.* 2009). However, the O-linked monosaccharide of the AniA glycoprotein is immunodominant and glycosylation of AniA is suggested to be an immune evasion mechanism in which the glycan moiety acts as a 'decoy' to diminish an antibody response against the functional region of the protein (Shewell *et al.* 2013). A truncated, non-glycosylated, form of AniA induces a non-native immune response against the conserved core region of the protein regardless of the glycosylation state and has been proposed as a vaccine antigen for *N. gonorrhoeae* (Shewell *et al.* 2013).

Additional glycoproteins have been reported for the pathogenic *Neisseria* via highly sensitive mass spectrometry methods (Vik *et al.* 2009; Anonsen *et al.* 2012). However, another study has suggested that for some of the additional proteins identified as glycoproteins, the majority of the protein expressed by the bacteria was essentially unglycosylated (Schulz *et al.* 2013).

INTERACTIONS OF THE PATHOGENIC *NEISSERIA* WITH HOST GLYCANS AND GLYCOPROTEINS

The pathogenic *Neisseria* express a variety of surface structures that interact with host cells. These include the major outer membrane proteins (e.g. pili, the opacity proteins Opc and Opa, and the major porins PorA and PorB) as well as other minor outer membrane structures and adhesins (Merz and So 2000; Virji, 2009). Studies have shown that some of the Neisserial surface structures bind to host glycans and glycoproteins; however, for the latter it is unclear as to whether these interactions are solely protein based, or are also glycan dependent (summarised in Table 3 and Fig. 1). Since these membrane proteins have often undergone post-translational modification by glycosylation, the binding interactions between these bacterial membrane proteins and the host may be either protein:protein, protein:glycan (Esko and Sharon 2009) or glycan:glycan based (Day *et al.* 2015). The pathogenic *Neisseria* are also known to interact with various other host glycans, such as gangliosides and oligosaccharides, although the specific Neisserial surface structures responsible for this binding are unknown (Table 3).

Opacity proteins (Opc and Opa)

The opacity proteins, Opc and Opa, are phase variable, integral, β-barrel outer membrane proteins involved in Neisserial adherence and invasion (Carbonnelle *et al.* 2009; Sa E Cunha, Griffiths and Virji 2010). Opc is not expressed by gonococci (Zhu, Morelli and Achtman 1999) and the *opcA* gene is absent in several meningococcal strains, including the majority of strains in the ST-11/ET-37 and ST-8/Cluster A4 clonal complexes (Seiler *et al.* 1996). When present in high levels, Opc aids in meningococcal adhesion and invasion into host cells (Virji *et al.* 1995). *Neisseria meningitidis* strains have 3–4 *opa* genes, while *N. gonorrhoeae* strains may express up to 11 *opa* genes (Bhat *et al.* 1991). Phase variation of *opa* genes is random and independent, so strains may express any of their repertoire of *opa* genes at any given time (Bhat *et al.* 1991). The structural variability displayed by these proteins has been linked to functional diversity between Opa variants (reviewed in Billker *et al.* 2000).

Opc and some Opa proteins can bind the glycosaminoglycans heparin and heparan sulfate proteoglycans (HSPGs) (van Putten and Paul 1995; De Vries *et al.* 1998). Heparin and heparan sulfate are both highly sulphated glycans, with heparin being found primarily in mast cell granules while heparan sulfate is found in plasma, the extracellular matrix and on cell surfaces of most mammalian cells (Rabenstein 2002). Through its interaction with HSPGs, Opc is able to support, but not sustain, meningococcal adhesion and invasion of host endothelial cells (Virji, Makepeace and Moxon 1994). Due to this and the fact that the interactions are of low affinity, this may not be the only ligand for the opacity proteins. Opc and a few of the Opa variants also interact with extracellular matrix glycoproteins such as vitronectin and fibronectin (Sa E Cunha, Griffiths and Virji 2010). These interactions facilitate association with endothelial cells via the endothelial integrins αVβ3-integrin and α5β1-integrin (the fibronectin and vitronectin receptors, respectively) and result in efficient cellular invasion by meningococci (Virji, Makepeace and Moxon 1994; Unkmeir *et al.* 2002b) and gonococci (Gómez-Duarte *et al.* 1997). Furthermore, vitronectin binding also increases meningococcal serum survival by inhibiting the insertion of membrane attack complex into the bacterium (Singh, Su and Riesbeck 2010). The interactions between the gonococcal Opa50 protein and vitronectin can be inhibited by heparin in dose-dependent manner, which suggests that this interaction may be glycan based (Gómez-Duarte *et al.* 1997).

Both Opa and Opc have been reported to bind a range of other carbohydrate structures including galactose and sialic acids (Moore *et al.* 2005). Sialic acid residues are typically found at the outermost end of glycan chains of all cell types (Varki 2008). They are a common terminal residue on mucins (Linden *et al.* 2008) and are an integral component of gangliosides which are abundantly expressed in neuronal tissues (Varki 2008). A majority of Opa proteins also recognise one or more members of the human CD66 carcinoembryonic antigen superfamily; a family of heavily glycosylated cell adhesion proteins which includes the carcinoembryonic antigen-related cell adhesion molecule family (Virji *et al.* 1996; Sadarangani, Pollard and Gray-Owen 2011). This interaction however is glycan independent (Bos *et al.* 1998).

Other adhesins that bind glycans

A range of other Neisserial surface antigens have been shown to bind host glycans or glycoproteins, and these are outlined in Table 3. These include the serogroup B vaccine antigen, the Neisserial heparin binding antigen (NHBA), which binds heparin

Table 3. Interactions of meningococcal and gonococcal structures with host glycans and glycoproteins.

Outer membrane structure	Species	Host glycan or glycoprotein	Role of interaction	Ref
Opc	Nm	Fibronectin, vitronectin	Invasion into brain endothelia	Unkmeir *et al.* (2002b), Sa E Cunha, Griffiths and Virji (2010)
		Heparin/HSPGs	Possible adhesin	De Vries *et al.* (1998)
		Various oligosaccharides including sialic acid	Adherence to epithelial cells	Moore *et al.* (2005)
Opa	Nm	Various oligosaccharides	Adherence to epithelial cells	Moore *et al.* (2005)
Msf	Nm	Vitronectin	Serum survival	Griffiths *et al.* (2011)
NHBA	Nm	Heparin	Serum survival	Serruto *et al.* (2010)
	Nm	HSPGs	Adherence to epithelial cells	Vacca *et al.* (2016)
fHBP	Nm	Factor H	Serum survival	Madico *et al.* (2006); Seib *et al.* (2009)
NhhA	Nm	Laminin	ND	Scarselli *et al.* (2006)
	Nm	HSPGs	ND	Scarselli *et al.* (2006)
	Nm	Heparan sulphate	ND	Scarselli *et al.* (2006)
NspA	Nm	Factor H	Serum survival	Lewis *et al.* (2010)
NadA	Nm	Integrins	ND	Nägele *et al.* (2011)
PorA	Nm	C4Bp	Serum survival	Jarva *et al.* (2005)
PorB	Nm	TLR2-TLR1 heterodimer	Immune evasion	Massari *et al.* (2002)
	Nm	Factor H	Serum survival	Lewis *et al.* (2013)
Pili	Nm	CD46	Unknown	Gill and Atkinson (2004)
	Nm	PAFr	Adherence to human airway cells	Jen *et al.* (2013)
	Ng	CR3	Adherence to cervical epithelial cells	Jennings *et al.* (2011)
Unknown adhesin	Nm	Gangliosides	Adherence to red blood cells	Rumiantsev *et al.* (1990)
	Nm	ECM components	ND	Eberhard *et al.* (1998)

Nm, *Neisseria meningitidis*, Ng, *Neisseria gonorrhoeae*. HSPG, heparan sulfate proteoglycans; ECM, extracellular matrix; C4Bp, C4 binding protein; TLR, Toll like receptor; PAFr, platelet activating factor receptor; CR3, complement receptor 3. ND, not determined.

(Serruto *et al.* 2010) and heparan sulfate proteoglycans (Vacca *et al.* 2016) via an arginine-rich region. NHBA binding to heparin mediates increased serum resistance (Serruto *et al.* 2010), potentially via interactions between heparin and factor H or C4b binding protein (Sahu and Pangburn 1993; Serruto *et al.* 2010). NHBA is also involved in meningococcal adherence to epithelial cells, via binding to heparan sulfate proteoglycans (Vacca *et al.* 2016). Other minor adhesins such as NadA, Msf, NspA and NhhA also bind host glycans and/or glycoconjugates (see Table 3).

CONCLUSION

Understanding the glycobiology of the pathogenic *Neisseria* is important for a better understanding of Neisserial pathogenesis, and may ultimately aid improved therapeutic and vaccine development. Surface glycans of the pathogenic *Neisseria* play a key role in resistance to host immune responses by reducing the efficiency of bacterial recognition and killing by the host, and by immune evasion via phase variation and molecular mimicry. These pathogens also harness the host glycocalyx, which provides opportunities for adherence that are key in colonisation (reviewed in Rouphael and Stephens 2012). The full range of glyco-interactions remains to be discovered. A recent study reports that terminal sugars on bacterial LPS/LOS mediate interactions with host cells via direct binding to host glycans (Day *et al.* 2015), and this may also be the case in the pathogenic *Neisseria* and is the subject of current investigation in our laboratory.

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